Polymorphisms of Adrenergic Cardiovascular Control Genes are Associated with Adolescent Chronic Fatigue Syndrome

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Abstract

Background

Chronic fatigue syndrome (CFS) is characterized by alterations of adrenergic cardiovascular regulation. We explored the presence of polymorphisms in adrenergic cardiovascular control genes in adolescent with CFS, and their relation to cardiovascular variables.

Methods

DNA from 52 CFS patients were analysed for 5 single nucleotide polymorphisms (SNPs) in genes encoding the Catechol-O-methyltransferase (COMT) (rs4680), the β_2-adrenergic receptor (rs1042713, rs1042714), the β_1-adrenergic receptor (rs1801253) and the α_{2a}-adrenergic receptor (rs1800544). Frequencies were compared to a reference population constructed from the NCBI database. Furthermore, associations between autonomic cardiovascular responses during a 20^o head-up tilt-test and polymorphisms frequencies within the patient group were explored.

Results

For the COMT SNP at rs4680, CFS patients had a higher frequency of the AA genotype (Met/Met) and a lower frequency of the G (Val) containing genotypes (AG and GG), as compared to the reference sample (p=0.046). Also, among CFS patients, the AA genotype was associated with lower diastolic blood pressure (DBP) at baseline (p=0.028) and a smaller increase in LF/HF (and index of cardiac sympathovagal balance) during head-up tilt (p=0.045) as compared to the AG/GG genotypes. For the rs1042714 SNP in the β_2-adrenergic receptor gene, CFS patients had a lower frequency of the GG genotype and a higher frequency of the genotypes containing C (CG and CC) (p=0.044). No associations to cardiovascular variables were found.

Conclusion

Genetic susceptibility to CFS might be related to polymorphisms of COMT and the β_2-adrenergic receptor. The importance of polymorphisms in other adrenergic cardiovascular control genes should be explored in further studies.

Introduction

The Chronic fatigue syndrome (CFS) is a common and disabling disease, characterized by severe fatigue and accompanying symptoms such as musculosceletal pain, unrefreshing sleep, sore throat, tender lymph nodes, concentration problems and headache (1). The underlying disease mechanisms are largely unknown. Previously, we have reported elevated levels of epinephrine and norepinephrine at rest among adolescent CFS patients as compared with healthy controls (2). Also, as compared to controls, CFS patients had higher blood pressures, heart rate and LF/HF ratio (an index of sinus node sympathicovagal balance, derived from spectral analyses of heart rate) at rest, and a stronger increase in these variables upon orthostatic stress (3, 4, 5). Other studies suggest an association between catecholamine levels and CFS symptoms such as fatigue and pain sensitivity (6,7). Taken together, these studies indicate enhanced sympathetic nervous activity in CFS, possibly contributing to the underlying pathophysiology (8).

Twin studies indicate a moderate heritability of CFS (9), and molecular analyses suggest an association to polymorphisms in genes involved in the metabolism and functioning of catecholamines (10). Interestingly, similar associations have been documented in disorders having several clinical features in common with CFS, such as the postural orthostatic tachycardia syndrome, fibromyalgia and chronic pain syndromes (11, 12, 13, 14). Furthermore, genetic polymorphisms are known to influence cardiovascular variables. For instance, polymorphisms in the β_2-receptor are associated with individual variations in heart rate, blood pressure, cardiac output and vasodilatation, in dynamic as well as in resting states (11, 15, 16, 17), whereas certain polymorphisms of the β_1-receptor are related to vasovagal syncope (18).

The aim of this study was to explore further the possible relation between polymorphisms of adrenergic cardiovascular control genes and adolescent CFS. We

hypothesized that polymorphism frequencies of genes encoding the adrenergic receptor proteins α_{2a}, β_1, and β_2, and the catabolic enzyme Catechol-O-methyltransferase (COMT) would be different among CFS adolescents as compared to a reference population. Furthermore, we hypothesized an association between these polymorphisms and variables indicating altered sympathetic cardiovascular control within the group of CFS patients.

Methods

Subjects

Patients with CFS ranging from 11 to 18 years of age were consecutively recruited from the paediatric outpatient clinic at Rikshospitalet University Hospital, Oslo, Norway, serving as a national referral centre for children and adolescents with unexplained chronic fatigue. Other disease states that might explain their present symptoms, such as autoimmune, endocrine, neurologic, or psychiatric disorders, were ruled out by a thorough and standardized set of investigations. Different case definitions of CFS exist. This study used a slight modification of the definition from the Centers for Disease Control and Prevention, in which the main criterion is more than 6 months of chronic or relapsing fatigue, severely affecting daily activities. In addition, according to this definition, patients should report at least 4 of 8 specific accompanying symptoms (headache, muscle pain, joint pain, sore throat, tender lymph nodes, impaired memory or concentration, unrefreshing sleep, and malaise after exertion). However the validity of this definition has been criticized in adults (19, 20) and children (21). Participation in this study required only 4 months of chronic or relapsing fatigue and no accompanying symptoms.

One week prior to the experiments, all participants were instructed not to drink beverages containing alcohol or caffeine, not to take any drugs, and not to use tobacco products. On the day of the experiments, they were requested to fast overnight.
Written informed consent was obtained from all participants and their parents. The study was approved by the Regional committee for ethics in medical research in Norway.

Genotyping

The NCBI SNP database (22) was used to assign SNP numbers; COMT SNP Rs4680, β2-receptor SNPs Rs1042714 and Rs1042713, β1-receptor SNP Rs1801253 and alpha2a SNP

Rs1800544 was selected for testing. Genomic DNA from whole blood cells was extracted using DNA isolation kit (E.Z.N.A. Blood DNA kit, Omega Bio-Tek, Inc., Norcross, Georgia, USA) and used for 5 exonuclease TaqMan PCR procedure. The genotyping was carried out using pre-designed TaqMan assays (Applied Biosystems, Foster City, USA) according to the manufacturer's recommendations. Approximately 10 ng genomic DNA was amplified in a 5 µl reaction mixture in a 384-well plate containing 1x universal TaqMan master mix and 1x assay mix, the latter containing the respective primers and MGB-probes labeled either with FAM or VIC. After initial denaturation and enzyme activation at 95°C for 10 min, the reaction mixture was subjected to 40 cycles of 95°C for 15 s and 60°C for 1 min. The reactions were performed on an ABI 7900HT sequence detection system. Negative controls containing water instead of DNA were included in every run. Genotypes were determined using the SDS 2.2 software (Applied Biosystems, USA).

Reference sample

A reference sample of the relevant genotypes was constructed from previously reported frequencies of European cohort collected from the NCBI SNP database (22). The reference frequencies were calculated as a weighted mean of the frequencies reported in the included studies. The weighing was done according to number of subjects in each study. In the reference sample for the COMT SNP at position rs 4680, four studies were included (23), for the β1 receptor SNP at rs 1801253 two studies were included (24), for both the β2-receptor SNPs at rs 1042713 and 1042714 three studies were included (25, 26) and one study was included in the reference sample for the α2a SNP at Rs 1800544 (27).

Head-up tilt-test

Head up tilt test (HUT) was performed according to the same protocol as was applied in previous CFS studies in our laboratory (3). The subjects lay supine on an electronically operated tilt table with foot-board support. They were attached to the Task Force Monitor® (Model 3040i, CNSystems Medizintechnik, Graz, Austria), a combined hardware and software device for non-invasive recording of cardiovascular variables (28). A 5 minutes baseline recording was obtained, after which the subjects were head-up tilted 20^{o} for 15 minutes. Instantaneous heart rate, arterial blood pressure and stroke volume were obtained from the ECG, right middle finger photoplethysmography and thoracic impedance, respectively; the latter is not reported in this article.

The RR-interval (RRI) from the heart rate recording was subjected to spectral analysis using an adaptive autoregressive algorithm, creating a time-varying spectrum (29). Spectral power densities (absolute values) were calculated in the low-frequency (LF) band (0.04-0.15 Hz) and the high-frequency (HF) band (0.15-0.4 Hz); we also calculated the LF/HF ratio. This indices are measures of autonomic heart rate control: The HF-variability of RRI is considered an index of vagal (parasympathetic) modulation of heart rate, whereas the LF-variability of RRI is due to the combined effect of cardiac vagal and sympathetic activity; the LF/HF-ratio is a measure of "sympathovagal balance" (30).

For each experimental run of HUT, the median of all cardiovascular variables were computed in the following epochs: 270 - 30 seconds before tilt (Baseline), and 30 - 270 seconds after tilt (Tilt). We also computed Δ-values (i.e. Tilt – Baseline).

Statistical analyses

The statistical analyses were performed using the SPSS (version 17.0, Inc, Chicago, IL) statistical package and Microsoft Excel for Mac 2008 (version 12.2.3). For each selected SNP, genotypic frequencies were dichotomized in CFS patients and the constructed reference

population, respectively, in order to increase statistical strength. Comparisons between the two groups were carried out using chi-square tests. Relations between polymorphisms and cardiovascular variables were explored by subgrouping the patients according to genotype, and then computing the median of each cardiovascular variable within each genotypic subgroup; as some of the cardiovascular variables deviated from a normal distribution, these medians were compared using the non-parametric Wilcoxon-Mann-Whitney's test. A *p*-value of 0.05 was considered statistically significant. Relations between polymorphisms and cardiovascular variables were only investigated for the SNPs with CFS genotype frequencies differing significantly from the genotype frequencies of the reference population.

Results

53 patients with CFS were included in the study, 40 % men, 11-18 years of age (Table 1) with cardiovascular characteristics as described in table 2. Significant differences in genotype frequencies between CFS patients and the reference samples were found for the COMT SNP at Rs 4680 and the β2-receptor SNP at rs 1042714 (Table 3). For the COMT SNP at Rs4680, the frequency of the AA genotype (Met/Met) was higher and the frequency of the G containing genotypes AG (Met/Val) and GG (Val/Val) was lower among CFS patients (p=0.046). For the β_2-adrenergic receptor SNP at rs1042714, the CFS patients had a higher frequency of the heterozygous genotype (CG) and lower frequency of the homozygous genotypes compared to the reference sample (p= 0.003). At the same locus the GG genotype appeared with a lower, and the C containing genotypes (CG and CC) with a higher frequency among the CFS patients as compared to the reference sample (p=0.044). A non-significant difference between the CFS patients and the reference sample was also found for the β_2-adrenergic receptor SNP at position Rs 1042713. CFS patients had a higher frequency of the GG genotype and a lower frequency of the CG/CC genotypes as compared to the reference sample (p=0.079).

Within the CFS group, the AA genotype of the rs4680 SNP in the COMT gene was associated with lower diastolic blood pressure (DBP) at baseline (p=0.028) and a smaller increase in LF/HF during tilt (p=0.045) as compared to the AG/GG-variants (Table 4). For the rs1042714 SNP in the β_2-adrenergic receptor gene, no significant associations between genotypes and cardiovascular variables were discovered (Table 5).

Discussion

The most important finding of this study is the high frequency of the AA genotype of rs4680 SNP in the COMT gene among CFS patients. COMT has a vital role in the degradation of catecholamines such as norepinephrine and epinephrine (31). The A allele encodes a substitution of valine to methionine (Val158Met), and those homozygous for this allele (met158met carriers) have a 3-to 4-fold reduction in catecholamine degrading enzymatic activity as compared to val158val carriers (32, 33). Attenuation of enzyme activity leads to higher concentrations of catecholamines, possibly amplifying the effect of sympathetic nervous activity. This is perfectly in line with our previous findings of high levels of norepinephrine and epinephrine among CFS patients, as well as a tendency towards enhanced sympathetic cardiovascular control during orthostatic stress (2,3,4,5). Also, the finding confirms results from explorative genetic studies indicating an association between CFS and the COMT gene (10). Interestingly, the low-efficiency (COMT) enzyme is also prevalent among patients with fibromyalgia, a chronic pain disorder having several clinical features in common with CFS (12, 13, 14). Thus, the AA genotype of rs4680 SNP in the COMT gene might constitute a genetic predisposition in both CFS and fibromyalgia.

Given the functional consequences of the AA genotype (met158met carriers), our findings of lower baseline DBP and Δ LF/HF (sympathetic response during tilt) among homozygous CFS patients were surprising. However, these results are in line with a recent report from Hagen and co-workers documenting that the GG genotype (val158val carriers) is associated with higher prevalence of increased systolic blood pressure compared to the AA and AG genotypes (34). One possible explanation might be that reduced break-down of catecholamines stimulates central inhibitory mechanisms, thus causing an attenuation rather than stimulation of sympathetic activity.

The CFS patients showed a lower frequency of the GG genotype of the β_2-adrenergic

receptor SNP at rs1042714 (p=0.044) (Table 3). The G allele implies that glutamine is replaced by glutamate at amino acid position 27 (26). Several phenotypes have been associated with this Gln27Glu polymorphism, but the functional effects remain unclear (35). Still, previous investigations suggest that polymorphisms of the β_2-adrenergic receptor gene are associated with conditions having several clinical features in common with CFS, such as temporomandibular disorder (characterized by chronic pain) (15) and the postural orthostatic tachycardia syndrome (POTS) (11), complying with our results. The non-significant difference of genotype frequencies found for the β_2-adrenergic receptor SNP at rs1042713 also imply that this receptor could play a role in the pathophysiology of CFS.

Taken together, this study suggests that genetic susceptibility to CFS is related to polymorphisms of adrenergic cardiovascular control genes, supporting a theory of altered sympathetic nervous activity as an important element in the underlying pathophysiology (8). Further studies should explore details of the molecular mechanisms, as well as other elements of the sympathetic signalling and catabolic system, such as the enzyme monoamine oxidase (MAO). As several of these elements are available for pharmacological influence, such studies might be of direct clinical relevance.

Study limitations

The subjects included in the reference population constructed from the NCBI database might differ in age and origin from the adolescent CFS population studied. Ideally, a control group of healthy adolescents from the same geographic area should have been assembled. Furthermore, the CFS population included only 53 subjects, resulting in very low frequencies for some genotypes and weakening the statistical power.

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Tables

Table 1 Subject characteristics	
Number of subjects	53
Females	32
Males	21
Age at the time of examination[1]	15 (2)
Height (cm)[1]	167 (11)
Weight (kg)[1]	57 (14)
[1] ( ) = standard deviation	

Table 2 Physiological characteristics of the subjects			
	Median	Min	Max
Baseline heart rate	69	44	95
Change of heart rate	3	-6	20
Baseline systolic blood pressure	109	93	133
Change of systolic blood pressure during HUT[1]	2	-5	12
Baseline diastolic blood pressure	67	50	84
Change of diastolic blood pressure during HUT[1]	3	-8	14
Baseline mean arterial blood	81	64	100
Change of mean arterial blood pressure during HUT[1]	3	-5	12
Baseline LF/HF (index of cardiac sympathovagal balance)	0.67	0.07	3.07
Change of LF/HF (index of cardiac sympathovagal balance)	0.18	-0.56	2.48
FSS score (index of fatigue; 5 = severe fatigue, 1 = no fatigue)	4.86	2,86	5
Days absent from shool weekly	3	2	5
[1]Head up tilt test (20° head up tilt for 15 minutes)			

Table 3- Genotype frequencies			
Rs4680_COMT2 (Val58Met)	GG+AG	AA	No
CFS	31	19	50
CFS frequency	0.62	0.38	1
Reference (NCBI)	114	35	149
Reference frequency	0.76	0.24	1
p-value Chi square testi CFS vs NCBI			0.046
Rs1801253 β1-receptor (Gly389Arg, C=Arg, G	CC+CG	GG	No
CFS	45	3	48
CFS frequency	0.94	0.06	1
Reference (NCBI)	78	5	83
Reference frequency	0.94	0.06	1
p-value Chi square testi CFS vs NCBI			0,95
Rs1042714 β2-receptor (Gln27Glu, C=Gln, G	CC+CG	GG	No
CFS	44	7	51
CFS frequency	0.86	0,14	1
Reference (NCBI)	81	25	106
Reference frequency	0.72	0.28	1
p-value Chi square testi CFS vs NCBI			0.044
Rs1042714 β2-receptor (Gln27Glu, C=Gln, G	CG	CC+GG	No
CFS	30	21	51
CFS frequency	0.59	0.41	1
Reference (NCBI)	45	61	106
Reference frequency	0.34	0.66	1
p-value Chi square testi CFS vs NCBI			0.003
Rs 1042713 β2-receptor (Arg16Gly, A=Arg, G	GG	AG/AA	No
CFS	15	33	48
CFS frequency	0.31	0.69	1
Reference (NCBI)	49	57	106
Reference frequency	0.46	0.54	1
p-value Chi square testi CFS vs NCBI			0.079
Rs 1800544 - α2a-receptor	GG	CG+CC	No
CFS	3	49	52
CFS frequency	0.06	0.94	1
Reference (NCBI)	0	22	22
Reference frequency	0	1	1
p-value Chi square testi CFS vs NCBI			0.25

Table 4 – Physiological variables related to genotypes of COMT[1] rs4680

	GG/AG	AA	p-value
	(n=31)	(n=18)	(Wilcoxon)
HR baseline	72,1	65,4	0,157
Δ[2] HR	3,2	3,2	0,626
SBP baseline	109,5	110	0,661
Δ SBP	2,8	2,7	0,922
DBP baseline	68,4	62,4	0,028
Δ DBP	4,3	4,7	0,252
LF-RRI baseline	516	731	0,495
Δ LF-RRI	-35	-92	0,367
HF-RRI baseline	670	1143	0,232
Δ HF-RRI	-107	-270	0,071
LF/HF baseline	0,72	0,72	0,591
Δ LF/HF	0,27	0,09	0,045

1)Catecholamine-methyl-transferase (COMT)

2)change in the variable during head-up-tilt (Δ)

Table 5 Physiological variables and genotypes the β-adrenerg receptor rs1042713

	CC/CG	GG	p-value
	(n=41)	(n=7)	(Wilcoxon)
HR baseline	71,1	62,7	0,427
Δ [1]HR	3,1	3,5	0,380
SBP baseline	109,6	107,4	0,380
Δ SBP	2,1	3,1	0,269
DBP baseline	66,8	68,5	0,813
Δ DBP	4,0	5,6	0,269
LF-RRI baseline	646	651	0,967
Δ LF-RRI	-98	111	0,350
HF-RRI baseline	906	1525	0,513
Δ HF-RRI	-245	-215	0,835
LF/HF baseline	0,73	0,43	0,335
Δ LF/HF	0,19	0,37	0,989

1)Change in the variable during head-up-tilt (Δ)